

EXHIBIT 46

Presence of Talc in Pelvic Lymph Nodes of a Woman With Ovarian Cancer and Long-Term Genital Exposure to Cosmetic Talc

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BACKGROUND: Although epidemiologic studies suggest talc use may increase ovarian cancer risk, there is no proof that talc used externally reaches the pelvis.

CASE: A 68-year-old woman with stage III ovarian papillary serous carcinoma revealed she had used talc daily for 30 years to powder her genital area. Examination of her pelvic lymph nodes under polarized light microscopy showed diffuse areas of birefringence compatible with talc, confirmed by scanning electron microscopy and X-ray spectroscopy.

CONCLUSION: This description of talc in pelvic lymph nodes of a woman with ovarian cancer and decades of exposure to talc may prompt new studies and offer new insights into the biologic basis for the consistent, but debated, association between talc use and ovarian cancer.

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An epidemiologic association between the use of cosmetic talc in genital hygiene and ovarian cancer was first described in 1982, and many subsequent studies found talc use to increase risk for ovarian cancer.¹ However, the causality of the relationship has been challenged for several reasons.²

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First, the association is a relatively weak one (ie, summary relative risk of approximately 1.3). Second, no clear increase in risk with duration of use has been found in most studies. Third, the ability of talc used in the genital area to enter the pelvic cavity has not been conclusively proven. At the time of pelvic surgery for ovarian cancer, pelvic lymph nodes are commonly sampled for staging purposes, but pathologic examination of the nodes is focused on the presence or absence of metastatic disease. More careful examination of pelvic lymph nodes from women with ovarian cancer may contribute to new perspectives in the debate regarding the role of talc in the causation of ovarian cancer, as illustrated by the following case.

CASE

A 68-year-old, married woman presented with abdominal swelling. A computed tomographic scan revealed a 13-cm pelvic mass, and her serum CA 125 level was more than 1,000. She was referred to the Gynecologic Oncology Service at the Brigham and Women's Hospital, where cytoreductive surgery was performed, including total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and pelvic lymph node sampling. A stage III papillary serous carcinoma with a minor clear cell component was found. Metastatic serous carcinoma was described in two of six right external iliac and obturator nodes. Postoperatively, the patient was referred for chemotherapy. She also consented to our interview about risk factors for ovarian cancer. This study is approved by the Dana Farber–Harvard Cancer Center Institutional Review Board and permits administration of general and dietary questionnaires, blood donation, and investigation of surgical specimen(s) after written informed consent. The patient's past history included three term deliveries followed by a tubal ligation. She had not smoked, used oral contraceptives, or used postmenopausal hormone therapy other than 6 months of progesterone therapy to regulate periods around the time of menopause, which occurred at age 50. There is a family history of colon cancer in a sister and maternal grandmother. At our interview, the patient stated she had used talc daily for 30 years as a body powder on the perineum and also applied it to underwear and sanitary napkins.

In searching for ideas to help clarify the association between talc use and ovarian cancer, we consulted with an expert on mesothelioma (J.G.), who pointed out that asbestos and other particulate material commonly migrates to lymph nodes.^{3,4} We decided that a more systematic examination of pelvic lymph nodes from ovarian cancer cases might be in order, beginning with this case. In examining the patient's pelvic lymph nodes, no distinct particulates were seen under regular light microscopy, although a diffuse histiocytic reaction was noted, even in a node without metastases (Fig. 1A). Under polarized light, diffuse



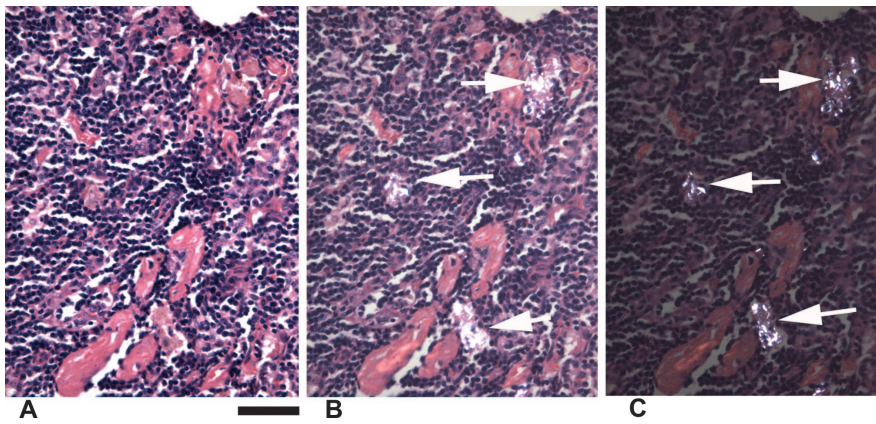


Fig. 1. Hematoxylin and eosin–stained section of a lymph node from the case showing morphologic findings with no polarization of the microscope light and with combinations of polarized and incident light at several different levels. **A.** Nodal morphology is illustrated and reveals no particulates seen without polarized light, but clusters of histiocytes are more prominent than usual. **B.** This panel shows the same field with polarized light plus additional light to view tissue context; birefringence is noted especially in areas of histiocyte clusters. *Arrows* are used to call attention to the birefringent particles. **C.** This shows the same field without added light, revealing the particulate nature of the birefringent material. *Arrows* highlight the particulate. Magnification bar shows 100 μm and applies to all three panels.

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birefringence was seen corresponding to the areas of histiocyte infiltration (Fig. 1B). Figure 1C shows the same field under polarization with no added light, revealing the particulate nature of the material, compatible with talc. Three of this patient's four nodes (not containing metastases) displayed polarizing material. Using methods described by Shelburne et al,⁵ we next examined lymph nodes from this patient by combined scanning electron microscopy and energy dispersive X-ray spectroscopy. Scanning electron microscopy revealed plate-like particulates in the 5–10 μm range within the lymph node, in which energy dispersive X-ray spectroscopy showed a magnesium and silicate signature—compatible with talc (Fig. 2A,B). Dys-trophic calcium deposits were also found within her nodes, probably a consequence of nodal aging. Of nodes from the next 12 patients examined, this case was strongest for

birefringence; but these nodes have not yet been subjected to scanning electron microscopy or energy dispersive X-ray spectroscopy. Figure 3 illustrates a node negative for polarization (or histiocyte reaction) from a patient with ovarian cancer who had not used talc.

COMMENT

Talc is a hydrous magnesium silicate chemically similar to asbestos but structurally quite different. Asbestos has a fiber-like structure and talc a plate-like one. Because of this difference, it has been argued that the relationship between asbestos and mesothelioma should not be invoked to explain how talc might cause ovarian cancer. However, one feature of expo-

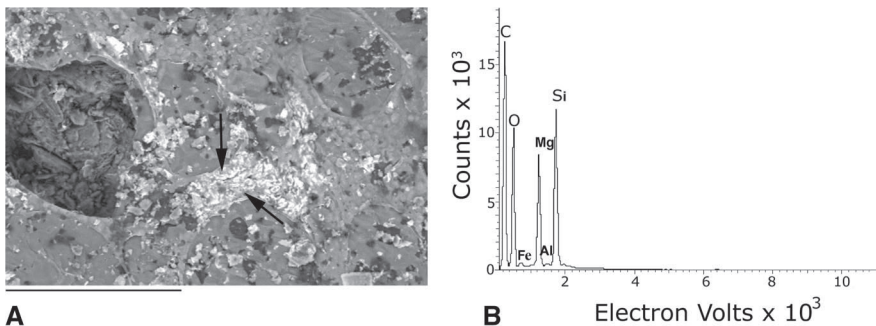


Fig. 2. Analytical microscopy. **A.** Scanning electron microscopy of a histologic section of the lymph node from the case shows a large collection of plate-like particulates in the 5–10 μm range (*arrows*) as well as scattered individual particulates. Magnification bar shows 100 μm . **B.** X-ray spectrum taken from the central bright area with particles reveals a Magnesium (Mg), Silicon (Si), and Oxygen (O) signature compatible with talc. A Carbon (C) signal is coming from the tissue or the underlying Carbon plancette or both.

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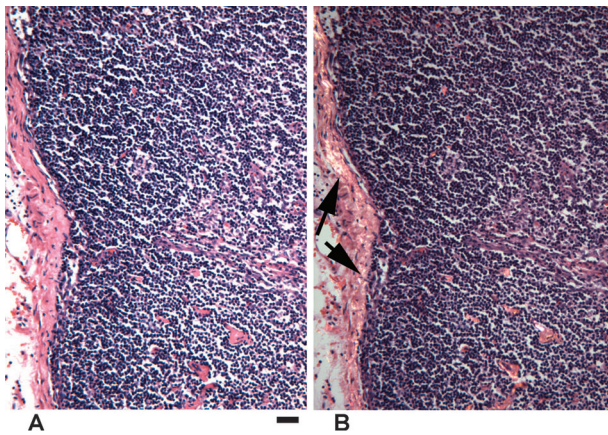


Fig. 3. Comparative node section illustrated from a woman reporting no talc use. **A.** Hematoxylin and eosin stained section showing fewer macrophages than seen in the node in Figure 1A. Magnification bar shows 100 μ m. **B.** Polarized light examination of the same area of the node showing only some birefringence in the node capsule (arrows) compatible with collagen.

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sure that the minerals do have in common is nodal dissemination. Migration and entrapment in lymph nodes is observed in human asbestos exposure and correlates with the asbestos burden.³ Talc has also been described in pulmonary lymph nodes of talc miners.⁴ However, a MEDLINE search of (all language) publications between January 1950 and February 2007 using the search terms, “talc,” “birefringence,” “histiocytosis,” “lymph nodes,” and “ovarian neoplasms,” revealed no reports of talc in lymph nodes of ovarian cancer patients.

In one of the few studies in women to evaluate the potential for talc to migrate into the pelvis, Heller et al studied normal ovaries from women having oophorectomy for benign disease.⁶ The protocol involved a multistep process of tissue rehydration, blotting, drying, digestion, rehydration, centrifugation, and multiple washes. After this process, polarizing bodies were found in all ovarian specimens examined by light microscopy. By electron microscopy, tissues from 5 of 12 women who regularly used talc and 6 of 12 who had not were found to have particles consistent with talc. The investigators concluded that talc can be found in ovaries but that this does not correlate with genital talc use. Contamination that might have been introduced during extensive processing is a potential weakness of this study.

In this case report, we describe examination of pelvic lymph nodes from a woman with ovarian cancer who had been a long-term talc user. Particles compatible with talc were clearly visible under polar-

ized light in regular hematoxylin and eosin-stained sections from her pelvic nodes, which were then shown by scanning electron microscopy and energy dispersive X-ray spectroscopy to be talc. Thus, as opposed to the aforementioned study, we focused on pelvic lymph nodes rather than ovaries; and talc was shown to be present in macrophages within the actual tissue, ruling out contamination during processing.

In reporting this case, we are not proposing that pelvic lymph nodes from women with ovarian cancer must now be subjected to electron microscopy. However, pathologists may wish to examine pelvic lymph nodes with evidence of histiocytic infiltrates by polarized light microscopy. Clear evidence of polarization may be reported so that clinicians can obtain information about potential talc exposure, if this information has not already been collected. Also we are not claiming that a causal relationship between ovarian cancer and talc use is proven for this case or in general. Because case reports cannot establish causality, we have begun a more extensive study of nodes with two purposes. First it is necessary to establish in a quantitative manner the likelihood of finding talc in lymph nodes of women with ovarian cancer and correlate this by whether they did or did not use talc. Second, studies of immune markers in nodes may help make the case for a causal connection.

What we do hope this case report accomplishes is to infuse a fresh perspective on the talc and ovarian cancer association. Previous biologic arguments linking talc and ovarian cancer have been based upon: similarities between talc and asbestos, the ability of talc to reach the ovaries through the open female tract, and induction of a mesothelioma-like cancer from the ovarian epithelium. Our new perspective would not depend upon structural similarities between talc and asbestos. The adverse effects of talc may relate to its ability to induce an inflammatory reaction, a well-established property of talc, independent of any similarity to asbestos.⁷ Also, we don't believe that talc needs to reach the ovaries to affect ovarian cancer risk; rather, the harmful effects of talc may involve inflammatory reactions in the lower genital tract, including the upper vagina, cervix, and endometrium. These tissues express the surface glycoprotein human mucin 1, MUC1, whose function is to protect cells from environmental stressors. It is likely that chronic talc exposure is one factor that upregulates MUC1 expression. Human mucin 1 is related to CA 125 (MUC16), and like CA 125 is overexpressed in ovarian cancer. It is known that women with ovarian cancer who have anti-MUC1 antibodies survive longer, leading us to propose that



many risk factors for ovarian cancer may be explained by their ability to raise or lower MUC1 immunity.⁸ Looking at predictors of anti-MUC1 antibodies, talc use was a factor that lowered anti-MUC1 antibodies. Thus, rather than a direct carcinogenic effect on ovarian epithelium, immune dysregulation involving MUC1 may be induced by chronic talc use that may lower protective immunity. Furthermore, sequestration of talc in nodes may affect antigen processing and be another important element in the postulated immune dysregulation.

In conclusion, this description of talc in pelvic lymph nodes of a long-term talc user with ovarian cancer may begin to reshape understanding about the relationship between talc and ovarian cancer and shed new light on whether talc used externally in the genital area is capable of migrating into the pelvis.

REFERENCES

1. Cramer DW, Liberman RF, Titus-Ernstoff L, Welch WR, Greenberg ER, Baron JA, et al. Genital talc exposure and risk of ovarian cancer. *Int J Cancer* 1999;81:351-6.
2. Wehner AP. Cosmetic talc should not be listed as a carcinogen: comments on NTP's deliberations to list talc as a carcinogen. *Regul Toxicol Pharmacol* 2002;36:40-50.
3. Friedrichs KH. Electron microscopic analyses of dust from the lungs and the lymph nodes of talc-mine employees. *Am Ind Hyg Assoc J* 1987;48:626-33.
4. Roggli VL, Benning TL. Asbestos bodies in pulmonary hilar lymph nodes. *Mod Pathol* 1990;3:513-7.
5. Shelburne JD, Estrada H, Hale M, Ingram P, Tucker JA. Correlative microscopy and microprobe analysis in pathology. In: Bailey GW, editor. *Proceedings of the 47th annual meeting of the Microscopy Society of America*. Vol 900. San Francisco (CA): San Francisco Press; 1989.
6. Heller DS, Westhoff C, Gordon RE, Katz N. The relationship between perineal cosmetic talc usage and ovarian talc particle burden. *Am J Obstet Gynecol* 1996;174:1507-10.
7. van den Heuvel MM, Smit HJ, Barbierato SB, Havenith CE, Beelen RH, Postmus PE. Talc-induced inflammation in the pleural cavity. *Eur Respir J* 1998;12:1419-23.
8. Cramer DW, Titus-Ernstoff L, McKolanis JR, Welch WR, Vitonis AF, Berkowitz RS, et al. Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1125-31.

Postpartum Sudden Death From Pulmonary Hypertension in the Setting of Portal Hypertension

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BACKGROUND: Pulmonary arterial hypertension carries a high maternal mortality rate in the peripartum period. Pulmonary hypertension may arise as a complication of portal hypertension with poor patient survival.

CASE: A young primigravida with chronic autoimmune hepatitis and portal hypertension presented at 26 4/7 weeks of gestation with contractions and bleeding. Within 48 hours, an 892-g female fetus was delivered vaginally without complications. On postpartum day 2, the mother was found on the floor by her bed. Although

initially responsive, within minutes she was unresponsive and resuscitation was unsuccessful. Postmortem examination showed cirrhosis and plexogenic pulmonary arteriopathy.

CONCLUSION: Increased awareness of pulmonary hypertension as a complication of portal hypertension and a high index of clinical suspicion are necessary to diagnose pregnant women with this condition and provide appropriate prenatal counseling and peripartum intervention.

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Pulmonary hypertension is an under-recognized complication of portal hypertension. We present an individual with known autoimmune hepatitis with cirrhosis and portal hypertension where underlying pulmonary hypertension was identified after her postpartum sudden death. Pulmonary hypertension may present in a subtle manner, but is important to appreciate in this high-risk obstetric patient population.

CASE

A young primigravida with a 10-year history of autoimmune hepatitis with chronic thrombocytopenia presented to the hospital at 26 4/7 weeks of gestation with contractions and bleeding. Before her pregnancy, she was a noncompliant transplantation candidate not using birth control. Prenatal care had been initiated at 6 weeks of

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